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Prevalence and Level of *Escherichia coli* O157:H7 in Feces and on Hides of Feedlot Steers Fed Diets with or without Wet Distillers Grains with Solubles†

J. E. WELLS,* S. D. SHACKELFORD, E. D. BERRY, N. KALCHAYANAND, M. N. GUERINI, V. H. VAREL, T. M. ARTHUR, J. M. BOSILEVAC, H. C. FREETLY, T. L. WHEELER, C. L. FERRELL, AND M. KOOHMARAIE‡

U.S. Department of Agriculture, Agricultural Research Station, U.S. Meat Animal Research Center, Clay Center, Nebraska 68933-0166, USA

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ABSTRACT

The objective of this study was to determine if wet distillers grains with solubles (WDGS) from corn in diets affected *Escherichia coli* O157:H7 in growing and finishing cattle; steers ($n = 603$) were randomly assigned to diets with or without WDGS. Hide and fecal samples were collected monthly (October through June) from each animal for enumeration and enrichment of *E. coli* O157:H7. In the growing phase (0 or 13.9% WDGS diets), fecal prevalence for *E. coli* O157:H7 in steers fed a diet with WDGS was twice that of the prevalence in control steers ($P < 0.001$). In the finishing phase (0 or 40% WDGS diets), the average prevalence in feces ($P < 0.001$) and on hides ($P < 0.001$) was higher for cattle fed WDGS. The average percentage of fecal *E. coli* O157:H7 enumerable samples during the finishing phase for cattle fed WDGS was 2.7% compared with 0.1% for control steers ($P < 0.001$). The average percentage of *E. coli* O157:H7 enumerable hide samples was not different between diets, but the cattle fed WDGS had higher levels ($P < 0.05$) of the pathogen. Animals fed WDGS had higher levels of *E. coli* ($P < 0.001$), higher pH values ($P < 0.001$), and lower concentrations of L-lactate ($P < 0.001$) in feces than those values of the control steers. These results indicate that feeding 40% WDGS could increase the level and prevalence of *E. coli* O157:H7 in and on feedlot cattle when *E. coli* O157:H7 is seasonally low.

Escherichia coli O157:H7 is a pathogen associated with many foods including undercooked ground beef, and in humans can cause illness and, in some cases, death. This pathogen asymptomatically colonizes the digestive tract of cattle *(1),* is shed in the feces, and contaminates the hides *(20).* During hide removal, some pathogens could be transferred to the carcass, and postharvest interventions have been implemented to reduce this contamination *(17).*

Beef cattle historically have been fed corn grain to improve quality and gain, but other feedstuffs such as wet distillers grains with solubles (WDGS) may be fed, with little detriment to growth *(16).* WDGS is a co-product from grain-based ethanol production, and in recent years, the ethanol production industry has competed for corn supply; thus, the use of WDGS as feed has increased. Recent studies have examined the effects on shedding of *E. coli* O157:H7 by cattle fed WDGS *(13, 14, 21),* but these reports differ in their results; in particular, the effects on shedding by cattle fed high levels of WDGS are entirely unclear.

After years of decline in incidence and outbreak numbers, the reported incidences of *E. coli* O157:H7 in the United States increased in 2007 *(7).* The U.S. Food Safety and Inspection Service tests and monitors *E. coli* O157:H7 in ground beef and trim samples, and the percentage of positive sample has increased each year from 2006 to 2008 *(27).* The beef industry and scientific community are actively investigating the basis for these shifts in *E. coli* O157:H7. Although circumstantial, recent increased use of WDGS in feedlot cattle diets has been speculated as a factor. If feeding WDGS to cattle increases *E. coli* O157:H7, then there should be increased prevalence and increased numbers of the pathogen in feces and possibly on hides. The objective of this study was to determine if feeding WDGS to feedlot cattle affects *E. coli* O157:H7 levels and prevalence in feces and on hides.

MATERIALS AND METHODS

Animals and diets. All animal procedures were reviewed and approved by the U.S. Meat Animal Research Center (USMARC) Animal Care and Use Committee.

A total of 608 spring-born steers were identified after weaning for this project to determine the effect of WDGS in growing and finishing rations on pathogen load in naturally infected feedlot cattle. The steers were from the 2007 Germplasm Evaluation Program (crossbred cattle representing predominant breeds in the United States), MARC III (a four-breed composite of Pinzgauer, Red Poll, Hereford, and Angus cattle), and Angus populations at USMARC, and were weaned from 4 to 14 September 2007. The steers were blocked by dam line, sire line, sire (if known), and

* Author for correspondence: Tel: 402-762-4174; Fax: 402-762-4209; E-mail: jim.wells@ars.usda.gov.

† Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name by the U.S. Department of Agriculture implies no approval of the product to the exclusion of others that might also be suitable.

‡ Present address: IEH Laboratories and Consulting Group, Lake Forest Park, WA 98155, USA.

weaning weight, and assigned to one of the two dietary treatment groups: without WDGS, which were the control (CON) steers, and with WDGS.

From weaning until slaughter, animals were provided ad libitum access to their respective diets. During the postweaning period prior to the study (48 to 58 days), all animals were acclimated to the USMARC feedlot and fed an alfalfa hay–corn silage diet. All animals were medicated with chlortetracycline (5 g per head per day) in the feed for 5 days at the time of weaning and again immediately prior to the start of the study. The study was initiated on 29 October 2007, with 603 steers (261 ± 32 kg body weight, 165 ± 17 days of age) in eight pens of 75 to 77 steers per pen (four pens per dietary treatment). Steers were sorted to the pens on day 0, according to the blocking scheme described above. The CON steers were fed standard diets through the growing and finishing phases of production, whereas the WDGS animals were fed similar diets, except WDGS replaced 47% of the corn grain component in each production phase diet (see below). Animals were weighed on days 0, 28, 49, 77, 105, 133, 161, 189, 217, and 245. Steers were implanted with Synovex-S (Fort Dodge Animal Health, Overland Park, KS) on day 0 and with Revalor-S (Intervet, Millsboro, DE) on day 105. Four pens (two CON and two WDGS) were harvested on day 231, and the remaining four pens were harvested on day 253.

In the rations formulated for the growing-phase diets (Table 1), WDGS replaced corn grain to yield a diet with 13.9% WDGS on a dry matter (DM) basis. After sampling on day 0, all pens received assigned diets for day 49 until mid-December. Over a 28-day period from mid-December (day 49) to mid-January (day 77), the steers were transitioned from the growing diet to the finishing diet via three intermediate diets with corn, or corn and WDGS replacing corn silage. The animals received the finishing diets (0% CON or 40% WDGS, DM basis) (Table 1) from mid-January 2008 (day 77) until slaughter (day 231 or 253). The corn source was high-moisture corn from October 2007 through May 2008 (day 189) samplings. The supply of high-moisture corn was depleted by May 2008; therefore, high-moisture corn was replaced by dry-rolled corn (purchased from local supply), and diets were reformulated for DM content differences in corn source. These diets were fed for the full 28-day period prior to the next sampling (day 217). All diets were formulated to meet or exceed National Research Council recommendations for growing and finishing beef steers *(19)*.

TABLE 1. *Formulated rations and calculated nutrient content for growing- and finishing-phase diets with and without WDGS fed to steers during study, and calculated resulting energy values*

	Growing diets[a,b]		Finishing diets[b,c]	
Ration component	CON	WDGS	CON	WDGS
Ingredient (% of DM)				
Corn, grain	29.50	15.64	82.75	45.15
Corn silage	67.00	69.40	13.75	13.75
WDGS	0.00	13.86	0.00	40.00
Soybean meal	2.40	0.00	2.40	0.00
Urea	0.40	0.00	0.40	0.00
Limestone	0.57	0.97	0.57	1.04
Salt	0.062	0.062	0.062	0.000
Monensin premix[d]	0.030	0.030	0.030	0.030
Thiamine premix[e]	0.023	0.023	0.023	0.023
Vitamin supplement[f]	0.008	0.008	0.008	0.008
Trace mineral supplement[g]	0.007	0.007	0.007	0.000
Nutrient content, formulated (% of DM)				
DM	46.04	38.11	71.07	51.98
CP	10.90	11.55	11.86	17.86
Fat	3.52	4.32	4.05	6.32
ADF	21.21	26.14	6.83	19.48
NDF	32.79	38.25	13.89	27.38
Calcium	0.540	0.551	0.403	0.451
Phosphorus	0.277	0.286	0.293	0.339
Metabolizable energy (Mcal/kg)	2.843	2.822	3.27	3.26
Net energy for maintenance (Mcal/kg)	1.703	1.688	1.991	1.981
Net energy for grain (Mcal/kg)	1.081	1.068	1.337	1.330

[a] Fed days 0 through 49.
[b] Animals were stepped up to finishing ration with three transition rations, from days 49 through 77.
[c] Fed days 78 through 245.
[d] Rumensin 80 (Elanco Animal Health, Indianapolis, IN).
[e] Premix contains 88 g of thiamine per kg.
[f] Supplement provides 8,800,000 IU of vitamin A, 880,000 IU of vitamin D, and 880 IU of vitamin E per kg.
[g] Supplement contains 13% Ca, 12% Zn, 8% Mn, 10% Fe, 1.5% Cu, 0.2% I, and 0.1% Co.

Sample collection and bacterial analyses. Samples of feces and hides were collected from each steer on days 0, 28, 49, 77, 105, 133, 161, 189, 217, and 245. Each animal was restrained in a squeeze chute to collect the samples. The fecal sample (at least 10 g) was collected as a rectal grab sample, with a clean gauntlet glove (Nasco, Ft. Atkinson, WI) for each animal. Feces were immediately transferred to a clean, closable plastic bag (Envision, Wichita, KS) for transport to the laboratory. The hide sample was collected with a sterile sponge (Nasco) premoistened with 20 ml of sterile buffered peptone water (Difco, Becton Dickinson, Sparks, MD). An area of approximately 1,000 cm^2 behind the left shoulder was wetted with distilled water and then sampled by hand with a clean latex glove each time with 10 up-down strokes of the sponge (five strokes per each side of the sponge), and the sample sponge was returned to the sterile sponge bag. Samples were transported to the laboratory within 1 h after sampling for processing.

A 10-g (±0.1 g) amount of each fecal sample was placed in a sterile filter bag (Nasco) with 90 ml of sterile tryptic soy broth (TSB; Difco, Becton Dickinson) containing 100 mM potassium phosphate buffer (18 mM KH_2PO_4 and 82 mM K_2HPO_4, pH = 7.2; Sigma, St. Louis, MO) *(3)* and mixed well by hand massage. A 500-μl aliquot was removed to a sterile 2-ml Cluster Biotube (Simport Corp., Beloell, Quebec, Canada) for enumeration. For the hide sponge samples, samples were mixed by hand squeezing, a 250-μl aliquot was removed to a sterile 2-ml Cluster Biotube for enumeration, and then 80 ml of TSB was added and then mixed again by hand. All samples with added media were enriched by incubation for 2 h at 25°C, which was followed by 6 h at 42°C. Samples were then held at 4°C overnight for immunomagnetic separation.

To enumerate *E. coli* O157 in each sample (described previously in *(6)*), 50 μl of each fecal and hide aliquot was plated onto ntCHROMO157 agar (CHROMO157 agar, DRG International, Mountainside, NJ) supplemented with 20 mg/liter novobiocin

(Sigma) and 0.8 mg/liter potassium tellurite (Sigma) by using an Autoplate 4000 spiral plater (Spiral Biotech, Inc., Norwood, MA). Plates were air dried and incubated overnight at 42°C. Presumptive colonies (up to five per sample) were tested by latex agglutination by using Dry Spot *E. coli* O157 (Oxoid, Ltd., Basingstoke, UK). Agglutination-positive colonies were counted and confirmed by PCR assay for combination of O157, H7 flagella, intimin, and Shiga toxin 1 and 2 genes *(12)*.

To determine *E. coli* O157 prevalence, filter bags with enriched fecal samples and sponge bags with enriched hide samples were mixed by hand massage, and 0.5 ml was removed to an individual well in deep-well 96-well blocks. Each well was preloaded with 0.5 ml of phosphate buffered saline with Tween (PBS-Tween; Sigma) and 25 μl (for fecal samples) or 5 μl (for hide samples) of anti-O157 immunomagnetic beads (Invitrogen Corp., Carlsbad, CA). Magnetic beads were mixed with enriched sample for 15 min at room temperature, on a vibrating MicroMixer Mx4 (Sanko Junyaku Co., Tokyo, Japan). The beads were removed from the sample, washed twice in PBS-Tween, and eluted into 100 μl of PBS-Tween with a Kingfisher 96 robotic processor (Thermo Fisher Scientific, St. Louis, MO). A 50-μl aliquot of the bead-bacterial complex was plated onto ntCHROMO157 agar. Plates were incubated overnight at 37°C. Presumptive colonies were tested by latex agglutination by using DrySpot *E. coli* O157 (Oxoid, Ltd.). At least two positive colonies were picked per plate and confirmed by PCR assay as described above.

For enumeration of *E. coli,* the initial fecal aliquot (10^{-1} dilution) was serially diluted to 10^{-2} and 10^{-4} in buffered peptone water, and 50 μl of each diluted fecal aliquot was plated onto separate CHROMagar ECC agar (DRG International) with an Autoplate 4000 spiral plater. Plates were air dried and incubated overnight at 37°C. Bacterial counts for *E. coli* (blue colonies) were taken manually with a Quebec Colony Counter (Warner-Lambert Technologies, Inc., Buffalo, NY).

Fecal analyses and environmental data collection. Fecal samples were measured for pH, volatile fatty acids (VFA), and L-lactate on experimental days 189, 217, and 245. To measure pH, approximately 2 g of fresh fecal sample was placed into a glass tube with 2 ml of ice-cold distilled H_2O, mixed by vortex, and stored at 4°C for no more than 4 h. Samples were warmed to room temperature, and the pH was measured in the 1:1 diluted feces by using a 6-mm general purpose, gel-filled pH electrode (Broadley-James Corp., Irvine, CA) and a UB-10 pH meter (Denver Instruments, Arvada, CO). To inhibit microbial activity and prepare feces for L-lactate measurements, 10.0 ± 0.1 g of fresh feces was weighed into a 50-ml centrifuge tube, acidified with 10 ml of 0.5 N H_2SO_4, and mixed well. Fecal samples were centrifuged (2,000 × *g* for 20 min at 4°C), and the supernatant was decanted to 15-ml tube and stored at −20°C until analyzed for L-lactate and VFA. The L-lactate was measured with a membrane-immobilized enzyme system (YSI Model 27, Yellow Springs Instrument Co., Yellow Springs, OH) and VFA were measured with gas chromatography, as described previously *(29)*. The L-lactate concentrations were corrected for volume dilutions used during preparation for analysis.

Weather conditions were monitored at the USMARC feedlot at an on-site, 24-h, all-season weather station. Air temperature and relative humidity were measured with a Vaisala model HMP35C probe in a model 41002 radiation shield (Vaisala, Helsinki, Finland). The probe was mounted at a 2.5-m elevation, and data was read every 5 min, and an average every 15 min was logged by a CR10X data logger (Campbell Scientific, Logan, UT). Daily average temperature was determined by averaging all 15-min data points collected after midnight for the next 24 h.

Statistical analyses. Bacterial counts in feces were log transformed, based on the count, the plated volume, and dilution factor of sample, and reported as CFU per gram of feces. Bacterial counts in hides were log transformed, based on the count and the plated volume, and the counts are reported as CFU per 100 cm^2. Pathogen prevalence in each pen was determined as a proportion of positives to total samples for each sample day and reported as a percentage. The prevalence data were analyzed as a split plot in time, with "pen" as the experimental unit. An initial model was used with all interactions and, after step-down analyses, the final model included the effects of dietary treatment, time, and pen nested within dietary treatment. Dietary treatment was tested, with "pen nested within dietary treatment" as the error term. Least-squares means and pooled standard errors of the means are presented in the text or tables where appropriate. For all statistical analyses, differences were considered significant when the probabilities were less than 0.05, and were considered tendencies when the probabilities were less than 0.10 but greater than 0.05. Statistical analyses were conducted by using the "GLM" procedure in SAS (SAS Institute, Inc., Cary, NC), except for Fisher's exact analyses *(26)* and the Wilcoxon-Mann-Whitney rank-sum nonparametric test (KaleidaGraph, Synergy Software Reading, PA).

RESULTS

Levels and prevalence for *Escherichia coli* O157:H7. Fecal shedding of *E. coli* O157:H7 was similar on day 0 (late October 2007) across treatments for the pen groups (Table 2). The percentage of animals in pens on day 0 with enumerable *E. coli* O157:H7 (>200 CFU/g of feces) in fecal samples was 6.4 for CON and 4.0 for WDGS (P = 0.50). The day 0 fecal prevalence for *E. coli* O157:H7–positive samples was 6.7 for CON and 5.0 for WDGS (P = 0.63). Hide contamination on day 0 for *E. coli* O157:H7 was not different, with 9.8 and 11.6% (P = 0.88) of hide samples with enumerable counts (≥40 CFU/100 cm^2), and 55.3 and 54.5% (P = 0.86) of hide samples were positive for *E. coli* O157:H7 from CON and WDGS, respectively.

During the growing phase (samples from days 28, 49, and 77, from late November 2007 through mid-January 2008) when animals were fed diets with 0 or 14% WDGS, the average percentage of fecal samples enumerable for *E. coli* O157:H7 ranged from 0.45 to 5.1% in pens, and was not affected by dietary treatment (P = 0.19) (Table 2). The average percentage of fecal samples positive for *E. coli* O157:H7 ranged from 4.1 to 26.4% in pens, and WDGS pens had higher average percentages than CON pens had (17.8 versus 8.8%, P = 0.009) in the growing phase. In hide samples, the average percentage of hide samples with enumerable *E. coli* O157:H7 ranged from 0.0 to 10.6% in all pens, and did not differ between CON and WDGS pens (P = 0.16). During the growing phase, the average percentage of hide samples positive for *E. coli* O157:H7 ranged from 11.3 to 71.1% for the pens, and WDGS average percentages tended to be higher than those of CON pens (58.5 versus 42.8%, P = 0.09). No treatment-time interactions were detected for the growing phase of production.

TABLE 2. *Percentage of samples with enumerable levels and prevalence of* Escherichia coli *O157:H7 in feces and on hides of cattle fed diets with and without WDGS*

Sample	Diet[a]		SEM	*P* value
	CON	WDGS		
Feces, avg enumerable[b]				
Day 0	6.4	4.0	2.25	0.50
Growing phase	2.0	3.6	0.81	0.19
Finishing phase	0.1	2.7	0.36	0.0001
Feces, avg prevalence				
Day 0	6.7	5.0	2.39	0.63
Growing phase	8.8	17.8	2.05	0.009
Finishing phase	1.5	14.9	1.85	0.0001
Hides, avg enumerable[b]				
Day 0	9.8	11.6	6.61	0.88
Growing phase	1.7	7.4	2.67	0.16
Finishing phase	0.0	5.6	1.95	0.051
Hides, avg prevalence				
Day 0	55.3	54.5	6.61	0.86
Growing phase	42.8	58.5	6.04	0.09
Finishing phase	9.2	32.8	3.29	0.0001

[a] The formulated rations differed with each phase of production and are listed in Table 1 (on a DM basis). The CON diet was 29.5% corn in the growing phase, and it was 82.7% corn in the finishing phase. The WDGS diet was 13.9% WDGS and 15.6% corn in the growing phase, and then was 40% WDGS and 45% corn in the finishing phase.

[b] A sample from the animal was scored enumerable positive if the enumeration plate had one or more *E. coli* O157:H7 CFU positive by latex and PCR tests. The lowest level of detection for enumeration was 200 CFU/g of feces and 40 CFU/ml for hide.

During the finishing phase (samples from days 105, 133, 161, 189, 217, and 245, from mid-February 2008 through late June 2008) with animals fed diets containing 0 or 40% WDGS, the average percentage of fecal samples with enumerable *E. coli* O157:H7 ranged from 0.0 to 0.2% for CON pens and from 0.3 to 3.7% for WDGS pens, and the mean percentage for WDGS was higher than it was for CON pens (2.7 versus 0.1%, $P = 0.0001$) (Table 2). The average percentage of fecal samples positive for *E. coli* O157:H7 ranged from 0.3 to 2.0 for CON pens and from 1.9 to 24.5% for WDGS pens, and mean percentages for WDGS were higher than they were for CON pens (14.9 versus 1.5%, $P = 0.0001$) in the finishing phase. The average percentage of hide samples with enumerable *E. coli* O157:H7 was 0% for all CON pens, and ranged from 0.2 to 20.2% for WDGS pens, and mean percentages for WDGS tended to be higher than they were for CON pens (0 versus 5.6%, $P = 0.051$). During the finishing phase, the average percentage of hide samples positive for *E. coli* O157:H7 ranged from 2.9 to 14.5% for CON pens and from 20.6 to 56.9% for WDGS pens, and again, mean percentages for WDGS were higher than they were for CON pens (9.2 and 32.8%, $P = 0.0001$). No treatment-time interactions were observed in the finishing phase of production.

Levels of enumerable *E. coli* O157:H7 in feces ranged from the lower detection limit of the assay at 200 (2.30 log) CFU/g to 1.59×10^6 (6.20 log) CFU/g, and on hides ranged from the lower limit of 40 (1.60 log) to 6.00×10^4 (4.78 log) CFU/100 cm^2 during the 245-day study. In the growing phase at the feedlot, a total of 50 fecal and 81 hide samples had enumerable levels of *E. coli* O157:H7 (Table 3). The average levels of enumerable *E. coli* O157:H7 in feces during the growing phase were 5.43×10^4 (4.74 log) CFU/g and 1.49×10^5 (5.17 log) CFU/g for animals fed CON and WDGS diets, respectively, and were not different ($P = 0.17$). The distributions of these fecal *E. coli* O157:H7 counts were not affected by diet in the growing phase ($P > 0.30$). In contrast, average levels of enumerable *E. coli* O157:H7 for hide samples from animals fed WDGS were higher than those fed the CON diet ($P = 0.03$), and the distributions of these counts differed up to 960 CFU/100 cm^2 ($P < 0.02$) in the growing phase. In the finishing phase for steers, a total of 37 fecal and 83 hide samples had enumerable levels of *E. coli* O157:H7 (Table 4). The average levels of enumerable *E. coli* O157:H7 in the feces were 1.3×10^3 (3.11 log) per g of feces and 6.85×10^4 (4.84 log) per g of feces for animals fed CON and WDGS diets, respectively, and these average counts and their distributions were not different between diets in the finishing phase ($P > 0.30$). However, the distribution of enumerable *E. coli* O157:H7 counts from hide samples did differ up to 960 CFU/cm^2 ($P < 0.02$).

The effect of time was significant ($P < 0.05$) for the percentage of hide and fecal samples, with enumerable *E. coli* O157:H7 in both production phases (Fig. 1a and 1b). The percentage of *E. coli* O157:H7 prevalence on hides and in feces was always greater than the percentage of samples with enumerable *E. coli* O157:H7, and differed with time ($P < 0.05$). On day 0, the percentages of fecal samples positive for *E. coli* O157:H7 were nearly equal to the percentages with enumerable *E. coli* O157:H7. This was likely the result of an unplanned chlortetracycline treatment for bovine respiratory disease just prior to that sampling. After day 28, the various measures of *E. coli* O157:H7 decreased and were lowest for all treatments on day 105 in mid-February; this corresponded to a period of prolonged cold temperatures in south central Nebraska that kept pen surfaces frozen (Fig. 1c). Relative to day 105, the level and prevalence for *E. coli* O157:H7 recovered by day 133 in March after the pens had thawed, but decreased again during days 151 and 189. Average levels and prevalence for *E. coli* O157:H7 did not increase again until days 217 and 245 in June, just prior to harvest.

Fecal characteristics and *E. coli* O157:H7. Fecal samples collected on day 189 were analyzed for pH and for *E. coli* (Table 5). The corn used in all rations up to day 189 was high-moisture corn. Fecal pH on day 189 for the CON animals was 5.99, and this was lower ($P < 0.001$) than the 6.79 obtained for WDGS animals. Fecal counts for *E. coli* averaged 6.06 log CFU/g of feces for CON animals, and this was lower ($P < 0.001$) than the 6.28 log CFU/g

TABLE 3. *Average and distribution of enumerable counts for* Escherichia coli *O157:H7 in feces and on hides of growing steers fed diets with and without WDGS*[a]

Sample	Enumerable count	Growing diet		
		CON, no. (%)	WDGS, no. (%)	*P* value
Fecal positives				
Avg (CFU log/g)[b]		4.74	5.17	0.17[c]
Distribution	Total fecal positives	78 (100)	158 (100)	ND[d]
	$<10^2$ CFU/g	60 (77)	126 (80)	0.47[e]
	$\geq 10^2$ CFU/g	18 (23)	32 (20)	0.37
	$\geq 10^3$ CFU/g	12 (15)	27 (17)	0.48
	$\geq 10^4$ CFU/g	5 (6.4)	14 (8.9)	0.35
	$\geq 10^5$ CFU/g	1 (1.3)	5 (3.2)	0.35
	$\geq 10^6$ CFU/g	0 (0)	3 (1.9)	0.30
Hide positives				
Avg (CFU/100 cm^2)[b]		58.6	262.8	0.03[c]
Distribution	Total fecal positives	382 (100)	584 (100)	ND
	<40 CFU/100 cm^2	367 (96)	518 (88)	0.21[e]
	≥ 40 CFU/100 cm^2	15 (3.9)	66 (11.3)	0.00002
	≥ 80 CFU/100 cm^2	2 (0.5)	34 (5.8)	0.000002
	≥ 240 CFU/100 cm^2	0 (0)	14 (2.4)	0.0008
	≥ 480 CFU/100 cm^2	0 (0)	8 (1.4)	0.018
	≥ 960 CFU/100 cm^2	0 (0)	3 (0.5)	0.22
	$\geq 2{,}000$ CFU/100 cm^2	0 (0)	2 (0.3)	0.37

[a] Growing diets fed are listed in Table 1. CON, 0 WDGS; WDGS, 13.8 WDGS.
[b] Average enumerable counts were calculated only from samples with enumerable *E. coli* O157:H7.
[c] Enumerable data not normally distributed and analyzed with the Wilcoxon-Mann-Whitney rank-sum nonparametric test.
[d] ND, not determined.
[e] Distribution data were analyzed with Fisher's exact test, and are based on the number of total fecal positives within each treatment.

of feces measured for the WDGS animals. After the day 189 sampling, high-moisture corn in the diets was replaced by dry-rolled corn. Feces were sampled again and analyzed on days 217 and 245 for pH, *E. coli,* and lactate. Fecal pH for the CON animals was 5.72, and this was significantly ($P < 0.001$) lower than the 6.14 obtained for WDGS animals. Fecal counts of *E. coli* averaged 6.74 log CFU/g of feces for CON animals, and this was significantly ($P < 0.001$) lower than the 7.02 log CFU/g of feces measured for the WDGS animals. Fecal concentration of L-lactate for WDGS was less than one-half the amount for CON animals ($P < 0.001$), and total VFA in feces was 7.3% less as compared with the control ($P < 0.001$). The relationships between fecal pH and *E. coli* for both CON ($r = -0.15$, $n = 596$) and WDGS ($r = -0.31$, $n = 590$) animals were linear and different from zero ($P < 0.05$), but the correlations were not strong ($r^2 < 0.3$).

In fecal samples from CON animals on days 189, 217, and 245, few samples with *E. coli* O157:H7 were detected, and therefore, potential relationships with pH and *E. coli* could not be properly analyzed. In the fecal samples from WDGS animals (Table 6) on day 189 (rations with high-moisture corn), the pH was not different for samples positive or negative for *E. coli* O157:H7 (6.80 ± 0.08 versus 6.79 ± 0.02, respectively, $P = 0.90$). On day 189, the levels of *E. coli* in the fecal samples positive for *E. coli* O157:H7 were not different from the samples testing negative (6.31 ± 0.15 versus 6.28 ± 0.04 log CFU/g, respectively, $P = 0.086$). In the fecal samples from WDGS animals tested on days 217 and 245 (ration with dry-rolled corn), the pH was not different for samples with or without *E. coli* O157:H7 (6.14 ± 0.04 versus 6.14 ± 0.04, respectively, $P = 0.99$). On days 217 and 245, the level of *E. coli* was lower ($P = 0.033$) in the fecal samples with *E. coli* O157:H7 than in the samples testing negative (6.94 ± 0.04 versus 7.06 ± 0.03 log CFU/g, respectively), whereas fecal L-lactate was higher (1.87 ± 0.15 versus 1.39 ± 0.11 mM, $P = 0.0095$) and total VFA was higher (83.42 ± 1.27 versus 79.20 ± 0.84 mM, $P = 0.0083$).

DISCUSSION

Cattle are natural reservoirs for *E. coli* O157:H7, and they shed this pathogen in their feces. For a number of years, feedlot cattle in the United States have been fed predominantly corn diets and, until recently, few studies for *E. coli* O157:H7 in feedlot cattle have been reported with diets other than corn. In this study conducted from October 2007 until June 2008, the use of WDGS in cattle diets was associated with increased *E. coli* O157:H7 in feces and on hides of naturally infected feedlot cattle. The difference in *E. coli* O157:H7 levels and prevalence between animals fed corn or WDGS was greatest during the finishing phase, when WDGS was being fed at 40% (DM basis) of the diet. However, the 10-fold magnitude of this difference could be a result of low levels of *E. coli* O157:H7 in our pens of animals fed corn during the finishing phase.

TABLE 4. *Average and distribution of enumerable counts for* Escherichia coli *O157:H7 in feces and on hides of finishing steers fed diets with and without WDGS*[a]

Sample	Enumerable count	Finishing diet		*P* value
		CON, no. (%)	WDGS, no. (%)	
Fecal positives				
Avg (CFU log/g)[b]		3.11	4.84	0.51[c]
Distribution	Total fecal positives	20 (100)	198 (100)	ND[d]
	$<10^2$ CFU/g	18 (90)	163 (82)	0.46
	$\geq 10^2$ CFU/g	2 (10)	35 (18)	0.30[e]
	$\geq 10^3$ CFU/g	1 (5.0)	16 (8.1)	0.46
	$\geq 10^4$ CFU/g	0 (0)	7 (3.5)	0.99
	$\geq 10^5$ CFU/g	0 (0)	3 (1.5)	0.99
	$\geq 10^6$ CFU/g	0 (0)	0 (0)	
Hide positives				
Avg (CFU/100 cm^2)[b]		<40	1,737.8	ND
Distribution	Total fecal positives	129 (100)	494 (100)	ND
	<40 CFU/100 cm^2	129 (100)	411 (83)	0.109[e]
	≥40 CFU/100 cm^2	0 (0)	83 (17)	0.00002
	≥80 CFU/100 cm^2	0 (0)	42 (8.5)	0.00004
	≥240 CFU/100 cm^2	0 (0)	25 (5.1)	0.0027
	≥480 CFU/100 cm^2	0 (0)	17 (3.4)	0.018
	≥960 CFU/100 cm^2	0 (0)	11 (2.2)	0.076
	≥2,000 CFU/100 cm^2	0 (0)	6 (1.2)	0.25

[a] Growing diets fed are listed in Table 1. CON, 0 WDGS; WDGS, 40 WDGS.
[b] Average enumerable counts were calculated only from samples with enumerable *E. coli* O157:H7.
[c] Enumerable data not normally distributed and analyzed with the Wilcoxon-Mann-Whitney rank-sum nonparametric test.
[d] ND, not determined.
[e] Distribution data were analyzed with Fisher's exact test, and are based on the number of total fecal positives within each treatment.

In growing steers fed the CON diet (corn silage and corn grain) from October through January, the average fecal *E. coli* O157:H7 prevalence was 8.8%. In comparison, in animals fed WDGS at 13.9% (DM basis), with WDGS replacing nearly one-half of the corn grain in the diet, the fecal prevalence more than doubled. Cattle diets with lower levels of corn can increase the persistence of *E. coli* O157:H7, as shown in vitro with feces and manures *(28, 29)*. Greater persistence could result in more hide contamination, and there was a tendency for greater hide prevalence when WDGS was fed. More important, the average level and the distributions of *E. coli* O157:H7 counts were greater for hide samples when WDGS was fed to growing steers, and this might reflect greater survival and levels of the pathogen on the pen surface during the time of this study.

Several reports have been published regarding *E. coli* O157:H7 in finishing cattle fed diets similar to those used in our current study. A recent comprehensive epidemiological study of U.S. feedlots reported that feeding brewers grains, a product similar to distillers grains, resulted in approximately sixfold greater odds for detecting *E. coli* O157 in the feces *(9)*. A recent series of studies reported by Jacob et al. *(13, 14)*, feeding 25% distillers grain (wet or dry, on a DM basis) was reported to increase the prevalence of *E. coli* O157 in feces. In one study with 0 and 25% dried distillers grains, Jacob et al. *(13)* reported 3.6 and 9.0% average *E. coli* O157 prevalence for pen floor pats, respectively, over a 12-week period. In a second study with 0 and 25% WDGS *(14)*, a significant difference with individual animals samples was observed only on 1 of 2 sampling days. In contrast to the above studies *(13, 14)*, data reported by Peterson et al. *(21)* indicated that WDGS in cattle diets at levels of 0 to 50% had no effect on the probability of finding *E. coli* O157:H7 in feces, but dietary levels of 20 and 30% WDGS were associated with fewer *E. coli* O157:H7 at the terminal rectum relative to 0% WDGS in the diet. These authors did not observe significantly higher probabilities for *E. coli* O157:H7 at the terminal rectum with 40 and 50% WDGS diets as compared with a 0% WDGS diet, but the authors did report large variations with these diets.

In the finishing phase with feedlot cattle, we collected individual animal samples from all animals every 28 days from February through June. Feeding 40% WDGS resulted in higher levels and prevalence for *E. coli* O157:H7 in feces and higher prevalence on hides when compared with feeding corn-based diets. The design utilized the largest available research pens at the USMARC feedlot and allowed higher stocking rates than had other published preharvest studies with WDGS, thereby reducing possible fluctuations and variations associated with smaller pen groups. During the finishing period, we observed typical seasonality for *E. coli* O157:H7 prevalence and overall >8% fecal *E. coli* O157:H7 prevalence in animals of our pen groups. However, the average *E. coli* O157:H7 prevalence in the pens of steers fed predominantly corn was <2%, whereas the prevalence in pens of steers fed 40% WDGS was nearly

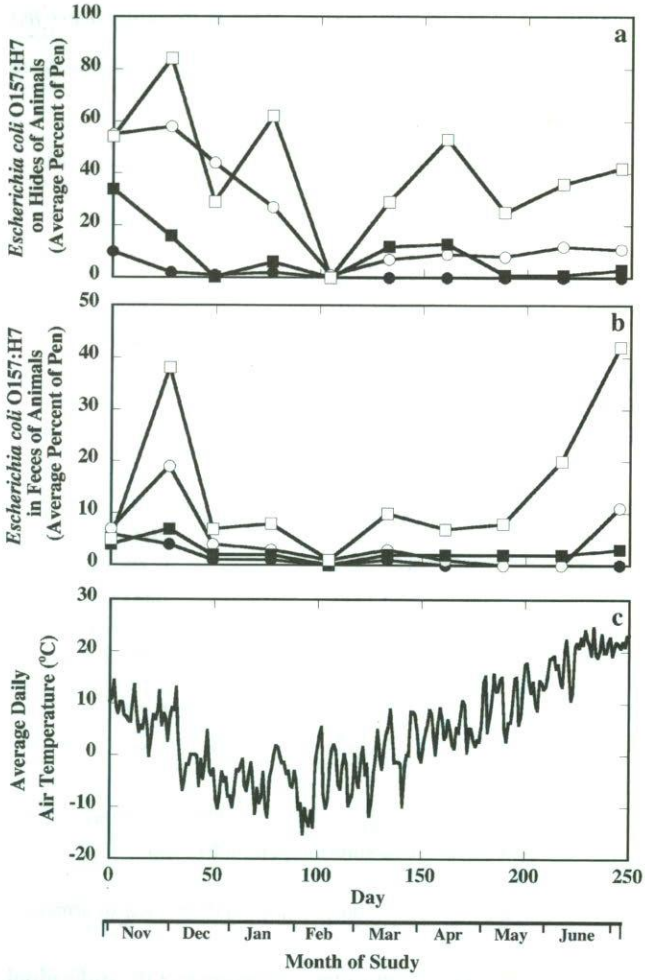

FIGURE 1. *Percentage of samples positive (open symbols) or enumerable (closed symbols) for* Escherichia coli *O157:H7 on hides (a), in feces (b), and on daily average temperature (c) over time of the study. Control diet (CON) pen groups are represented by circles (○, ●), and wet distillers grains with solubles diet (WDGS) pen groups are represented by squares (□, ■). The growing phase comprises days 1 to 77, and the finishing phase comprises days 78 to 245 of production. Each month of the study is denoted in the bar across the bottom of the figure.*

15%. The low average prevalence for our cattle fed corn was unexpected when compared with unpublished results from our feedlot, but it still was in line with seasonal observations at commercial slaughtering plants reported previously *(2)*. The difference in *E. coli* O157:H7 shedding between diets was such that the percentage of enumerable samples was greater for WDGS pens than the percent prevalence for the pens of cattle fed corn. It is unlikely that differences in *E. coli* O157:H7 shedding during the growing phase affected pathogen shedding in the finishing phase, since essentially no *E. coli* O157:H7 was observed with either dietary treatment on the first finishing-phase sampling in February. The February sampling occurred after a prolonged freeze, during which the average daily temperature did not exceed −10°C for nearly 2 weeks, and likely prevented cross-contamination within the pens.

Pen prevalence for *E. coli* O157 is a notoriously variable parameter at any point *(15, 23)*, and large variations may influence results. In the finishing phase, we detected very low prevalence on hides and in feces for all of the control pens, and it was not until the end of the study on day 245 that *E. coli* O157:H7 was appreciably detected in feces. In contrast, the 40% WDGS pens did have detectable

TABLE 5. *Fecal pH, (average counts of)* Escherichia coli, *L-lactate, and total VFA from finishing steers fed diets with and without WDGS*[a]

Parameter[b]	Finishing diet		*P* value
	CON	WDGS	
Fecal pH			
HMC in diet	5.99	6.79	0.0001
DRC in diet	5.72	6.14	0.0001
Fecal *E. coli* (log CFU/g)			
HMC in diet	6.06	6.28	0.0001
DRC in diet	6.74	7.02	0.0001
Fecal L-lactate (mM)			
HMC in diet	ND[c]	ND	
DRC in diet	3.72	1.56	0.0008
Fecal total VFA (mM)			
HMC in diet	ND	ND	
DRC in diet	87.05	80.68	0.0001

[a] Finishing diets fed are listed in Table 1. CON, 0% WDGS; WDGS, 40.0% WDGS.
[b] HMC, high-moisture corn (samples collected on day 189 of study); DRC, dry-rolled corn (samples collected on days 217 and 245 of study).
[c] ND, not determined.

TABLE 6. *Observed relationships between* Escherichia coli *O157:H7 in feces and fecal pH,* E. coli, *L-lactate, and total VFA for animals fed WDGS with component HMC*[a] *or DRC*[b]

Component	Fecal *E. coli* O157:H7 status		*P* value
	Positive	Negative	
HMC (day 189)			
No. of samples	23	277	
Fecal pH	6.80	6.79	0.90
Fecal *E. coli* (log CFU/g)	6.31	6.28	0.086
L-Lactate (mM)	ND[c]	ND	
Total VFA (mM)	ND	ND	
DRC (days 217 and 245)			
No. of samples	105	194	
Fecal pH	6.14	6.14	0.99
Fecal *E. coli* (log CFU/g)	6.94	7.06	0.033
L-Lactate (mM)	1.87	1.39	0.0095
Total VFA (mM)	83.42	79.20	0.0083

[a] High-moisture corn fed as corn grain component in all diets from day 105 until sampling on day 189.
[b] Dry-rolled corn fed as corn grain component in all diets starting after sampling on day 189 until harvest.
[c] ND, not determined.

E. coli O157:H7 on hides and in feces from day 133 until the end of the study. In the WDGS pens, one pen had higher levels and prevalence, particularly on the hides, than the other WDGS pens from day 133 through day 217, and might have contributed to an overestimate of treatment effects. However, removal of this pen from the data analysis did not eliminate the significance, although it did reduce the magnitude of the difference. In a study conducted over the same period but in a different year with smaller pen groups (30 to 32 animals), we have seen an enormous amount of variability in hide (mean, 6%; range, 0 to nearly 100%) and feces (mean, 26%; range, 0 to 94%) prevalence for *E. coli* O157:H7 within and among feedlot pens over time (unpublished data). In our current study, all WDGS pens had higher levels and prevalence for *E. coli* O157:H7 than had any of the control pens. Sanderson et al. *(22)* reported rapid increases in pen prevalence across pens in the summer months and hypothesized that close proximity of pens may have facilitated transmission. In our study, control pens that were negative adjoined WDGS pens that were positive for *E. coli* O157:H7 for repeated samplings. Nonetheless, the differences in magnitude of up to 10-fold might have been lower if we had more pens of animals available for study to better account for pen variation, or if we had conducted the study in summer when *E. coli* O157:H7 is normally higher.

Diet can affect the chemical composition of and certain bacterial populations in the feces. In previous work with cattle fed different diets, an inverse relationship between fecal pH and generic *E. coli* levels was observed for diets based on corn and corn silage as compared with hay *(4).* Considering that WDGS has very little starch *(24),* and in relation to the previous work *(4),* fecal pH was expected to have been higher and fecal levels of *E. coli* lower for animals fed diets with WDGS. Indeed, average fecal pH was significantly higher, but average fecal *E. coli* levels also were higher for the animals fed 40% WDGS compared with animals fed corn. The role of diet in changing *E. coli* populations is not well understood, but it is likely that *E. coli* and *E. coli* O157:H7 are populating similar niches and are utilizing similar nutrients *(10).* Hindgut environments that select for more *E. coli* may also result in more *E. coli* O157:H7.

We observed a wide range of pH values for feces from cattle fed diets with either 0 or 40% WDGS. Within the cattle fed diets with 40% WDGS, the presence or absence of *E. coli* O157:H7 in feces was not associated with differences in the fecal pH. This was true for day 189, when high-moisture corn was used in the diet, as well as for days 217 and 245, when dry-rolled corn was used. The observations made here corroborate a recent report by Depenbusch et al. *(8),* which indicate that fecal shedding of *E. coli* O157 is not related to fecal pH when feeding corn processed in different ways.

The levels of *E. coli* in feces of the cattle fed 40% WDGS were inversely related to fecal pH, such that at lower fecal pH, higher fecal levels of *E. coli* were observed. On day 189, with animals being fed the WDGS and high-moisture corn, fecal prevalence was less than 10%, and shedding of *E. coli* O157:H7 was not associated with levels of *E. coli* for the fecal samples. On days 217 and 245, for the same animals fed WDGS but with dry-rolled corn, prevalence was more than 20 to 40%, and shedding of *E. coli* O157:H7 was associated significantly with lower levels of *E. coli* in the feces. It is evident that higher levels of *E. coli* were found in feces of WDGS-fed cattle, but within that diet, animals that were shedding *E. coli* O157:H7 had lower levels of *E. coli.* In vitro experiments *(10)* have demonstrated that strains of *E. coli* O157:H7 and *E. coli* have similar fitness for growth, but more of the *E. coli* O157:H7 strains tested produced intraspecies inhibitory substances, and this may explain some of our in vivo observations.

Fox et al. *(11)* reported fecal prevalence for *E. coli* O157 was lower in animals fed dry-rolled grain than in animals fed steam-flaked grain. Theoretically, dry-rolled corn would have greater starch bypass into the colon, and fermentation would generate conditions less hospitable for *E. coli* O157:H7 to colonize and replicate. Depenbusch et al. *(8)* failed to see a relationship between pathogen shedding and fecal pH or starch concentration. Lactate, in particular L-lactate, has been shown to be inhibitory in vitro to *E. coli* O157:H7 *(18),* but no study has reported if fecal L-lactate is associated with pathogen shedding. Concentrations for L-lactate were measured in feces collected from days 217 and 245, when animals were fed dry-rolled corn, and in these samples, L-lactate was significantly higher for the cattle fed corn as compared with WDGS. However, the fecal concentrations for L-lactate averaged 3.72 mM for the cattle fed corn, well below the 50 to 200 mM inhibitory levels reported *(18),* and very few fecal samples had L-lactate concentrations >10 mM in our study.

In the feces from finishing cattle fed 40% WDGS, shedding of *E. coli* O157:H7 was associated with slightly elevated L-lactate concentrations (1.87 versus 1.39 mM). In animals that had shed the *E. coli* O157:H7 at least three times over the finishing phase, the fecal L-lactate concentrations were nearly threefold higher relative to animals that were never positive (data not shown). Additional L-lactate and VFA in the feces might be indicative of soluble nutrients being available in the lower gastrointestinal tract, and might provide *E. coli* O157:H7 colonized in the colon and rectum a source for carbon and energy. As a group, *E. coli* does not synthesize and excrete extracellular hydrolytic enzymes, and as such is unable to degrade starch and other polymers *(25).* Additional work needs to be done to support this hypothesis and determine specifically if fecal L-lactate, VFA, or another factor contributes to *E. coli* O157:H7 shedding in feces of cattle fed WDGS.

Feeding 40% WDGS to cattle in this study was associated with higher *E. coli* O157:H7 fecal shedding in the feedlot environment. However, at harvest, the hide is a significant source for *E. coli* O157:H7 transmission to the carcass *(2, 5, 20).* Throughout the finishing phase after the February sample collection, hide prevalence for *E. coli* O157:H7 was higher for the cattle fed diets with 40% WDGS. More important, in the finishing phase, we only

recovered hide samples with enumerable *E. coli* O157:H7 from the animals fed WDGS. In recent in vitro work, we have found that *E. coli* O157:H7 persistence was longer in manure from animals fed 40% WDGS compared with 0% *(28)*. Longer survival of *E. coli* O157:H7 in the feedlot, as well as in the lairage pen, could result in more *E. coli* O157:H7 on the hide at harvest.

In this study, feeding WDGS was associated with increased *E. coli* O157:H7 for feedlot cattle. In pens of finishing animals, the differences in all *E. coli* O157:H7 measures for 0 and 40% WDGS in the diet were significant. The magnitude of the difference may be a consequence of the low prevalence in the control pens in the late winter and early spring, and/or the higher levels in one WDGS pen. In the cattle fed diets with 40% WDGS, fecal shedding was not associated with fecal pH. Potential relationships between fecal *E. coli,* fecal L-lactate, and total VFA with fecal shedding of *E. coli* O157:H7 were observed in cattle fed WDGS as compared with corn. However, those relationships were inverted when examined within cattle fed 40% WDGS in the diet, and could be a consequence of bacterial competition. Nonetheless, it is not entirely clear why feeding high levels of WDGS to cattle results in more *E. coli* O157:H7, and a contributing factor could be the extended persistence of *E. coli* O157:H7 in the environment resulting in greater reinfection within the pen, in addition to fecal characteristics themselves.

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